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Date of mailing (day/month/year) 14 April 1999 (14.04.99)	
International application No. PCT/US98/15387	**Applicant's or agent's file reference** 96429/9008
International filing date (day/month/year) 24 July 1998 (24.07.98)	**Priority date** (day/month/year) 26 July 1997 (26.07.97)
Applicant FIRST, Neal, L. et al	

1. The designated Office is hereby notified of its election made:

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25 February 1999 (25.02.99)

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/00, A01K 67/027

A2

(11) International Publication Number: **WO 99/05266**

(43) International Publication Date: 4 February 1999 (04.02.99)

(21) International Application Number: PCT/US98/15387

(22) International Filing Date: 24 July 1998 (24.07.98)

(30) Priority Data:
60/053,103 26 July 1997 (26.07.97) US

(71) Applicant *(for all designated States except US)*: WISCONSIN ALUMNI RESEARCH FOUNDATION [US/US]; 614 Walnut Street, P.O. Box 7365, Madison, WI 53707–7365 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: FIRST, Neal, L. [US/US]; 3157 Highway 78, Mt. Horeb, WI 53572 (US). DOMINKO, Tanja [SL/US]; 2320 White Oak Trail, Oregon, WI 53575 (US). MITALIPOUA, Maissam [US/US]; 2130 University Avenue #6, Madison, WI 53705 (US).

(74) Agent: BARTA, Kent, S.; Michael Best & Friedrich LLP, 100 East Wisconsin Avenue, Milwaukee, WI 53202–4108 (US).

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Published
Without international search report and to be republished upon receipt of that report.

(54) Title: TRANS–SPECIES NUCLEAR TRANSFER

(57) Abstract

A method of producing cloned nuclear transfer embryos from differentiated donor cells is described. The method includes culturing differentiated donor cells in low serum medium and using bovine oocytes as recipient oocytes for donor cells from different species. This method may be used to produce genetically identical animals and transgenic animals.

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INTERNATIONAL SEARCH REPORT

Int ational application No.
PCT/US 98/15387

Box I Observations where ertain laims were f und unsearchabl (Continuation of item 1 f first heet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

B x II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-11 24 25 28

a method for producing nuclear transfer bovine embryos from bovine donor cells and bovine recipient oocytes comprising: inducing bovine donor cells to undergo G0 arrest; fusing said bovine donor cell to an enucleated bovine recipient oocyte to create a nuclear transfer embryo; and activating said nuclear transfer embryo; bovine embryo produced by this method; nuclear transfer bovine embryo comprising cytoplasm derived from a bovine oocyte, bovine cytoplasm derived from a differentiated bovine cell, cell membrane derived from a bovine oocyte, cell membrane derived from a differentiated bovine cell and nuclei derived from a differentiated bovine cell

2. Claims: 12-23 26 27 29

a method for producing nuclear transfer embryos from donor cells of one species and recipient oocytes from another species comprising: inducing the donor cells to undergo G0 arrest; fusing said donor cell to an enucleated recipient oocyte of another species to create a nuclear transfer embryo; and activating said nuclear transfer embryo; embryo produced by this method; nuclear transfer interspecies embryo comprising cytoplasm and cell membrane derived from one species and differentiated cytoplasm, differentiated cell membrane and nuclei derived from a differentiated cell from another species

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 98/15387

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/00 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 07669 A (ROSLIN INST EDINBURGH ;CAMPBELL KEITH HENRY STOCKMAN (GB); WILMUT) 6 March 1997 cited in the application	1-3,5,9, 11,28
Y	see page 2, line 26 - page 5, line 24 see page 7, line 13 - line 24; examples 1,4 see page 8, line 24 - page 9, line 12; claims	1-29

Y	STICE S L ET AL: "PLURIPOTENT BOVINE EMBRYONIC CELL LINES DIRECT EMBRYONIC DEVELOPMENT FOLLOWING NUCLEAR TRANSFER" BIOLOGY OF REPRODUCTION, vol. 54, no. 1, January 1996, pages 100-110, XP000671718 cited in the application see the whole document	1-3, 5-11,24, 25,28

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 February 1999	23.02.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chambonnet, F

4

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	SIMS M ET FIRST: "PRODUCTION OF CALVES BY TRANSFER OF NUCLEI FROM CULTURED INNER CELL MASS CELLS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 90, no. 13, June 1993, pages 6143-6147, XP000604993 cited in the application see the whole document	1-3,5-7, 10
Y	CHESNE P ET AL: "NUCLEAR TRANSFER IN CATTLE: BIRTH OF CLONED CALVES AND ESTIMATION OF BLASTOMERE TOTIPOTENCY IN MORULAE USED AS A SOURCE OF NUCLEI" LIFE SCIENCES, vol. 316, 1993, pages 487-491, XP000197855 see the whole document	1-3
Y	US 5 496 720 A (SUSKO-PARRISH JOAN L ET AL) 5 March 1996 cited in the application see the whole document	4-10
P,Y	WO 98 30683 A (UNIV MASSACHUSETTS A PUBLIC IN) 16 July 1998 see the whole document	3-11
A	WOLFE, B.A. ET AL.: "Embryos produced by the transfer of caprine nuclei to enucleated bovine oocytes are capable of cleavage but not to develop to blastocysts" BIOLOGY OF REPRODUCTION, vol. 50, no. suppl. 1, 1994 - 1972, page 72 XP002092259 see abstract 72	12
A	HEYMAN Y ET AL: "CLONING OF DOMESTIC SPECIES" ANIMAL REPRODUCTION SCIENCE, vol. 42, no. 1/04, 1996, pages 427-436, XP000671884 see page 429, paragraph 2.5	12
P,Y	WO 98 07841 A (UNIV MASSACHUSETTS) 26 February 1998 see the whole document	12-23, 26,27,29
T	ZAWADA, W.M. ET AL.: "Somatic cell cloned transgenic bovine neurons for transplantation in Parkinsonian rats" NATURE MEDICINE, vol. 4, no. 5, May 1998, pages 569-574, XP002092260 see the whole document	1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/US 98/15387

Patent document cited in search report	Publication date	Patent family member(s)	Publication dat
WO 9707669 A	06-03-1997	AU 6831096 A	19-03-1997
		CA 2229568 A	06-03-1997
		CZ 9800608 A	15-07-1998
		EP 0849990 A	01-07-1998
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/00, A01K 67/027

A3

(11) International Publication Number: **WO 99/05266**

(43) International Publication Date: 4 February 1999 (04.02.99)

(21) International Application Number: PCT/US98/15387

(22) International Filing Date: 24 July 1998 (24.07.98)

(30) Priority Data:
60/053,103 26 July 1997 (26.07.97) US

(71) Applicant *(for all designated States except US)*: WISCONSIN ALUMNI RESEARCH FOUNDATION [US/US]; 614 Walnut Street, P.O. Box 7365, Madison, WI 53707–7365 (US).

(72) Inventors; and

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(74) Agent: BARTA, Kent, S.; Michael Best & Friedrich LLP, 100 East Wisconsin Avenue, Milwaukee, WI 53202–4108 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
15 April 1999 (15.04.99)

(54) Title: TRANS–SPECIES NUCLEAR TRANSFER

(57) Abstract

A method of producing cloned nuclear transfer embryos from differentiated donor cells is described. The method includes culturing differentiated donor cells in low serum medium and using bovine oocytes as recipient oocytes for donor cells from different species. This method may be used to produce genetically identical animals and transgenic animals.

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Published
With international search report.
With amended claims and statement.

(88) Date of publication of the international search report:
15 April 1999 (15.04.99)

Date of publication of the amended claims and statement:
23 September 1999 (23.09.99)

(54) Title: TRANS–SPECIES NUCLEAR TRANSFER

(57) Abstract

A method of producing cloned nuclear transfer embryos from differentiated donor cells is described. The method includes culturing differentiated donor cells in low serum medium and using bovine oocytes as recipient oocytes for donor cells from different species. This method may be used to produce genetically identical animals and transgenic animals.

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AMENDED CLAIMS

[received by the International Bureau on 23 April 1999 (23.04.99);
original claims 1-29 replaced by amended claims 1-15 (3 pages)]

1. A method of producing nuclear transfer embryos from donor cells of one species and recipient oocytes from another species comprising:

inducing the donor cells to undergo G_0 arrest;

fusing said donor cell to an enucleated recipient oocyte of another species to create a nuclear transfer embryo;

and activating said nuclear transfer embryo.

2. The method of claim 1 wherein said G_0 arrest of donor cells is induced by culture in low serum medium.

3. The method of claim 1 wherein said donor cells are selected from the group consisting of embryonic derived cells, germ cells, somatic cells, and genetically modified cells.

4. The method of claim 1 wherein said enucleated recipient oocyte is an enucleated bovine recipient oocyte.

5. The method of claim 4 wherein said enucleated bovine recipient oocyte is selected from the group of bovine oocytes undergoing nuclear maturation within 16 hours of beginning in vitro culture.

6. The method of claim 1 wherein said enucleated bovine recipient oocyte and said donor cell are fused by electric pulse to form a nuclear transfer embryo.

7. The method of claim 1 wherein said fusion is performed 16-32 hours after the beginning of in vitro culture.

8. The method of claim 1 wherein said nuclear transfer embryo is activated by elevating intracellular calcium and the incubating with a serine threonine kinase inhibitor.

9. The method of claim 8 wherein intracellular calcium is elevated by incubation with ionomycin and the serine threonine kinase inhibitor is DMAP.

10. The method of claim 1 wherein said activation is 16-32 hours after the beginning of in vitro culture.

11. The method of claim 6 wherein said fusion is 16-52 hours after the beginning of in vitro culture.

12. An embryo produced by the method of claim 1.

13. A method of producing nuclear transfer embryos from a donor cell of one species and a bovine recipient oocyte comprising:

culturing non-bovine donor cells selected from the group consisting of embryonic derived cells, somatic cells, germ cells, and genetically modified cells in low serum medium so that said donor cells are induced to arrest in the G_0 stage of the cell cycle;

selecting a bovine recipient oocyte which has completed nuclear maturation before 16 hours from the beginning of in vitro culture;

enucleating said bovine recipient oocyte after 16-32 hours of in vitro culture;

placing said donor cell under the zone pellucida of said enucleated oocyte so that said donor cell contacts said enucleated oocyte;

fusing said donor cell with said enucleated oocyte by electric pulse at 16-32 hours after the beginning of in vitro culture to create a nuclear transfer embryo;

and activating said nuclear transfer embryo by sequential incubation with ionomycin and 6-dimethylaminopurine at 16 to 32 hours after the beginning of in vitro culture.

14. The embryo produced by the process of claim 13.

15. A nuclear transfer embryo comprising cytoplasm and cell membrane from one species and differentiated cytoplasm, differentiated cell membrane, and nuclei derived from a differentiated cell of another species.

STATEMENT UNDER ARTICLE 19

The original claims 1-29 have been amended, by canceling original claims 1-11, 24, 25, and the remaining original claims 12-23, 26, 27 and 29 have been renumbered as claims 1-15. The claims after amendment are now directed solely to a method for producing nuclear transfer embryos from donor cells of one species and recipient oocytes from another species.

CLAIMS

What is claimed is:

1. A method of producing nuclear transfer bovine embryos from bovine donor cells and bovine recipient oocytes comprising:

inducing bovine donor cells to undergo G_0 arrest;

fusing said bovine donor cell to an enucleated bovine recipient oocyte to create a nuclear transfer embryo;

and activating said nuclear transfer embryo.

2. The method of claim 1 wherein said G_0 arrest of donor cells is induced by culture in low serum medium;

3. The method of claim 1 wherein said bovine donor cells are selected from the group consisting of embryonic derived cells, germ cells, somatic cells, and genetically modified cells.

4. The method of claim 1 wherein said enucleated bovine recipient oocyte is selected from the group of bovine oocytes undergoing nuclear maturation within 16 hours of beginning in vitro culture.

5. The method of claim 1 wherein said enucleated bovine recipient oocyte and said bovine donor cell are fused by electric pulse to form a nuclear transfer embryo.

6. The method of claim 1 wherein said fusion is performed 16-24 hours after the beginning of in vitro culture.

7. The method of claim 1 wherein said nuclear transfer embryo is activated by elevating intracellular calcium and then incubating with a serine threonine kinase inhibitor.

8. The method of claim 7 wherein intracellular calcium is elevated by incubation with ionomycin and the serine threonine kinase inhibitor is DMAP.

9. The method of claim 1 wherein said activation is 16 to 32 hours after the beginning of in vitro culture.

10. The method of claim 5 wherein said fusion is 16-52 hours after the beginning of in vitro culture.

11. An embryo produced by the method of claim 1.

12. A method of producing nuclear transfer embryos from donor cells of one species and recipient oocytes from another species comprising:

inducing the donor cells to undergo G_0 arrest;

fusing said donor cell to an enucleated recipient oocyte of another species to create a nuclear transfer embryo;

and activating said nuclear transfer embryo.

13. The method of claim 12 wherein said G_0 arrest of donor cells is induced by culture in low serum medium.

14. The method of claim 12 wherein said donor cells are selected from the group consisting of embryonic derived cells, germ cells, somatic cells, and genetically modified cells.

15. The method of claim 12 wherein said enucleated recipient oocyte is an enucleated bovine recipient oocyte.

16. The method of claim 15 wherein said enucleated bovine recipient oocyte is selected from the group of bovine oocytes undergoing nuclear maturation within 16 hours of beginning in vitro culture.

17. The method of claim 12 wherein said enucleated bovine recipient oocyte and said donor cell are fused by electric pulse to form a nuclear transfer embryo.

18. The method of claim 12 wherein said fusion is performed 16-32 hours after the beginning of in vitro culture.

19. The method of claim 12 wherein said nuclear transfer embryo is activated by elevating intracellular calcium and the incubating with a serine threonine kinase inhibitor.

20. The method of claim 19 wherein intracellular calcium is elevated by incubation with ionomycin and the serine threonine kinase inhibitor is DMAP.

21. The method of claim 12 wherein said activation is 16-32 hours after the beginning of in vitro culture.

22. The method of claim 17 wherein said fusion is 16-52 hours after the beginning of in vitro culture.

23. An embryo produced by the method of claim 12.

24. A method for producing nuclear transfer bovine embryos from bovine donor cells and bovine recipient oocytes comprising:

culturing bovine donor cells selected from the group consisting of embryonic derived cells, somatic cells, germ cells, and genetically modified cells in low serum medium so that said donor cells are induced to arrest in the G_0 stage of the cell cycle;

selecting a bovine recipient oocyte which has completed nuclear maturation before 16 hours from the beginning of in vitro culture;

enucleating said bovine recipient oocyte after 16-32 hours of in vitro culture;

placing said donor cell under the zona pellucida of said enucleated oocyte so that said donor cell contacts said enucleated oocyte.

fusing said donor cell with said enucleated oocyte by electric pulse at 16-32 hours after the beginning of in vitro culture to create a nuclear transfer embryo;

and activating said nuclear transfer embryo by sequential incubation with ionomycin and 6-dimethylaminopurine at 16 to 32 hours after the beginning of in vitro culture.

25. The embryo produced by the process of claim 24.

26. A method of producing nuclear transfer embryos from a donor cell of one species and a bovine recipient oocyte comprising:

culturing non-bovine donor cells selected from the group consisting of embryonic derived cells, somatic cells, germ cells, and genetically modified cells in low serum medium so that said donor cells are induced to arrest in the G_0 stage of the cell cycle;

selecting a bovine recipient oocyte which has completed nuclear maturation before 16 hours from the beginning of in vitro culture;

enucleating said bovine recipient oocyte after 16-32 hours of in vitro culture;

placing said donor cell under the zone pellucida of said enucleated oocyte so that said donor cell contacts said enucleated oocyte;

fusing said donor cell with said enucleated oocyte by electric pulse at 16-32 hours after the beginning of in vitro culture to create a nuclear transfer embryo;

and activating said nuclear transfer embryo by

sequential incubation with ionomycin and 6-dimethylaminopurine at 16 to 32 hours after the beginning of in vitro culture.

27. The embryo produced by the process of claim 25.

28. A nuclear transfer embryo comprising cytoplasm derived from a bovine oocyte, bovine cytoplasm derived from a differentiated bovine cell, cell membrane derived from a bovine oocyte, cell membrane derived from a differentiated bovine cell and nuclei derived from a differentiated bovine cell.

29. A nuclear transfer embryo comprising cytoplasm and cell membrane from one species and differentiated cytoplasm, differentiated cell membrane, and nuclei derived from a differentiated cell of another species.

09/463276

REC'D 19 NOV 1999

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

18

Applicant's or agent's file reference 96429/9008	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US98/15387	International filing date *(day/month/year)* 24 JULY 1998	Priority date *(day/month/year)* 26 JULY 1997
International Patent Classification (IPC) or national classification and IPC IPC(6): A01K 67/027; C12N 15/00 and US Cl.: 800/15, 24		
Applicant WISCONSIN ALUMNI RESEARCH FOUNDATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   [✓] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV [ ] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25 FEBRUARY 1999	29 SEPTEMBER 1999
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JANET M. KERR JOYCE BRIDGERS PARALEGAL SPECIALIST CHEMICAL MATRIX Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (January 1994)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/15387

I. Basis of the report

1. This report has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments)*:

☐ the international application as originally filed.

☒ the description, pages (See Attached) , as originally filed.
pages _____ , filed with the demand.
pages _____ , filed with the letter of _____.
pages _____ , filed with the letter of _____.

☒ the claims, Nos. (See Attached) , as originally filed.
Nos. _____ , as amended under Article 19.
Nos. _____ , filed with the demand.
Nos. _____ , filed with the letter of _____.
Nos. _____ , filed with the letter of _____.

☒ the drawings, sheets/~~fig~~ (See Attached) , as originally filed.
sheets/~~fig~~ _____ , filed with the demand.
sheets/~~fig~~ _____ , filed with the letter of _____.
sheets/~~fig~~ _____ , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☒ the description, pages NONE.

☒ the claims, Nos. 16-29.

☒ the drawings, sheets/~~fig~~ NONE.

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:

NONE

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/15387

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citati ns and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims	1-11, 13	YES
	Claims	12, 14, 15	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-15	NO
Industrial Applicability (IA)	Claims	1-15	YES
	Claims	NONE	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-15 meet the criteria set out in PCT Article 33(4) for industrial applicability.

Claims 1-11 and 13 meet the criteria set out in PCT Article 33(2) for novelty.

Claims 12,14 and 15 lack novelty under PCT Article 33(2) as being anticipated by Wolfe et al (1990) Theriogenology 33, page 350.

Wolfe et al teach embryos and nuclear transfer embryos that where donor cell is from a different species that the recipient bovine enucleated oocyte (see Table). Wolfe teaches that four cleaved bovine-goat nuclear transplant embryos were obtained that contained nucleated cells and that of these one developed into a blastocyst (paragraph 2, lines 1-3). The method of making the embryos and nuclear transfer embryos does not provide a distinguishing feature over the embryos and nuclear transfer embryos taught by Wolfe et al. Thus, Wolfe et al clearly anticipates the claimed invention.

Applicants arguments have been considered but are not persuasive as the claimed embryos, obtained as a product by process, are not distinguishable over the reference embryos as both the reference embryos and the claimed embryos can be cultured up to the blastocyst stage.

Claims 1-11 and 13 lack an inventive step under PCT Article 33(3) as being obvious over Wolfe et al (1990) Theriogenology 33, page 350 in view of WO 97/07669 published 06 March 1997.

Wolfe et al teach methods of producing nuclear transfer embryos that where donor cell is from a different species that the recipient bovine enucleated oocyte, and fusion is by electric pulse (see Table). Wolfe teaches that four cleaved bovine-goat nuclear transplant embryos were obtained that contained nucleated cells and that of these one developed into a blastocyst (paragraph. 2, lines 1-3). '669 teaches the insertion of a quiescent cell or the nucleus from a quiescent cell, that is a G_0 cells caused by serum starvation, into the (Continued on Supplemental Sheet.)

Supplemental B x
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

I. BASIS OF REPORT:

THIS REPORT HAS BEEN DRAWN ON THE BASIS OF THE DESCRIPTION,
PAGES, 1-27, AS ORIGINALLY FILED.
PAGES, NONE, FILED WITH THE DEMAND.
AND ADDITIONAL AMENDMENTS:
NONE

THIS REPORT HAS BEEN DRAWN ON THE BASIS OF THE CLAIMS,
NUMBERS, NONE, AS ORIGINALLY FILED.
NUMBERS, NONE, AS AMENDED UNDER ARTICLE 19.
NUMBERS, NONE, FILED WITH THE DEMAND.
AND ADDITIONAL AMENDMENTS:
CLAIMS 1-15, FILED WITH THE LETTER OF 18 JUNE 1999

THIS REPORT HAS BEEN DRAWN ON THE BASIS OF THE DRAWINGS,
SHEETS, NONE, AS ORIGINALLY FILED.
SHEETS, NONE, FILED WITH THE DEMAND.
AND ADDITIONAL AMENDMENTS:
NONE

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (CONTINUED):
PERIVITELLINE SPACE OF AN ENUCLEATED OOCYTE TO PRODUCE NUCLEAR TRANSFER UNITS OR EMBRYOS (PAGE 8, LINES 14-22; PAGE 20, LINES 1-24 AND PAGE 21, LINES 11-16). '699 ALSO TEACHES THAT ACTIVATION INVOLVES INCREASES IN CALCIUM CONCENTRATIONS (PAGE 14, LINES 15-18). THUS GIVEN THE TEACHINGS OF WOLFE ET AL IN VIEW OF '669, IT WOULD HAVE BEEN OBVIOUS TO THE ORDINARY ARTISAN TO MAKE NUCLEAR TRANSFER EMBRYOS WHERE THE SPECIES OF THE RECIPIENT ENUCLEATED OOCYTE AND THE DONOR G_0 CELLS ARE OF DIFFERENT SPECIES. THE DETERMINATION OF OTHER PARAMETERS SUCH AS TIME OF FUSION, CULTURE CONDITIONS AND ACTIVATION METHODS WERE WELL WITHIN THE SCOPE OF SKILLS OF THE ORDINARY ARTISAN AT THE TIME OF FILING.

APPLICANTS' ARGUMENTS HAVE BEEN CONSIDERED BUT ARE NOT PERSUASIVE AS METHODS FOR GENERATING EMBRYOS WHERE THE DONOR CELL IS FROM A DIFFERENT SPECIES THAN THE RECIPIENT ENUCLEATED OOCYTE, AND METHODS FOR GENERATING EMBRYOS BY INSERTION OF A QUIESCENT CELL OR THE NUCLEUS FROM A QUIESCENT CELL, I.E., A CELL IN GO, INTO THE PERIVITELLINE SPACE OF AN ENUCLEATED OOCYTE TO PRODUCE EMBRYOS ARE KNOWN IN THE ART. AS BOTH METHODS PRODUCE EMBRYOS, THE SKILLED ARTISAN WOULD HAVE HAD A HIGH EXPECTATION OF SUCCESSFULLY PRODUCING NUCLEAR TRANSFER EMBRYOS WHERE THE SPECIES OF THE RECIPIENT ENUCLEATED OOCYTE AND THE DONOR GO CELLS ARE OF DIFFERENT SPECIES.

———— NEW CITATIONS ————

B. A. WOLFE ET AL. PREIMPLANTATION DEVELOPMENT OF EMBRYOS PRODUCED BY INTERGENERIC NUCLEAR TRANSPLANTATION. THERIOGENOLOGY. JANUARY 1990, VOL. 31, NO. 1, PAGE 350, SEE ENTIRE ARTICLE.

PATENT COOPERATION TREA. .

09/463276

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rul s 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
96429/9008		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 98/ 15387	24/07/1998	26/07/1997
Applicant		
WISCONSIN ALUMNI RESEARCH FOUNDATION et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [X] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/15387

B x I Ob ervations where certain claims w r f und un archabl (C ntinuation of it m 1 f first she t)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

B x II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-11 24 25 28

a method for producing nuclear transfer bovine embryos from bovine donor cells and bovine recipient oocytes comprising: inducing bovine donor cells to undergo G0 arrest; fusing said bovine donor cell to an enucleated bovine recipient oocyte to create a nuclear transfer embryo; and activating said nuclear transfer embryo; bovine embryo produced by this method; nuclear transfer bovine embryo comprising cytoplasm derived from a bovine oocyte, bovine cytoplasm derived from a differentiated bovine cell, cell membrane derived from a bovine oocyte, cell membrane derived from a differentiated bovine cell and nuclei derived from a differentiated bovine cell

2. Claims: 12-23 26 27 29

a method for producing nuclear transfer embryos from donor cells of one species and recipient oocytes from another species comprising: inducing the donor cells to undergo G0 arrest; fusing said donor cell to an enucleated recipient oocyte of another species to create a nuclear transfer embryo; and activating said nuclear transfer embryo; embryo produced by this method; nuclear transfer interspecies embryo comprising cytoplasm and cell membrane derived from one species and differentiated cytoplasm, differentiated cell membrane and nuclei derived from a differentiated cell from another species